IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Gruenberg, M.

Serial No.: 09/824,906
Conf. No. 9764

Filed: April 2, 2001

For: AUTOLOGOUS IMMUNE CELL THERAPY: CELL COMPOSITIONS, METHODS AND APPLICATIONS TO TREATMENT OF HUMAN DISEASE

Art Unit: 1644

Examiner: Unassigned

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:

Commissioner for Patents
Washington, D.C. 20231, on this date.

10/05/01
Date

Kelly Fischer

COPY

ATTACHMENT TO THE PRELIMINARY AMENDMENT MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)

IN THE SPECIFICATION:

Please amend the paragraph on page 11, lines 3-14, as follows;

Therefore, it is an object herein to provide such improved methods. In particular, it is an object herein to provide methods for expanding immune cells for use in adoptive immunotherapy protocols without the use of exogenous IL-2. It is also an object herein to provide methods to generate a large array of cell compositions, including compositions containing regulatory cells, for use in adoptive immunotherapy protocols.

It is an object herein to provide means to produce compositions containing clinically relevant numbers of such cells. [he] The availability of a an array of cell compositions permits the design of adoptive immunotherapy protocols for a wide variety of diseases and immune function alterations. Therefore, it is an object herein to provide methods for treating various disorders and altering immune function.

Please amend the paragraph on page 12, lines 1-8, as follows;

The compositions of regulatory cells provide a means to alter the immunoregulatory balance of a patient, either locally or [sytemically] systemically, by changing the predominant regulatory cell population. Because

many disease states occur with the loss of regulated balance of the immune system that is normally maintained by regulatory immune cells, the availability of clinically-relevant numbers of regulatory immune cells provides a means to correct these imbalances. This ability offers great potential for treating a variety of diseases.

Please amend the paragraph on page 23, lines 7-15, as follows;

As used herein, a hollow cell fiber culture system includes [of] a hollow fiber bioreactor as well as pumping means for perfusing medium through said system, reservoir means for providing and collecting medium, and other components, including electronic controlling, recording or sensing devices. A hollow fiber bioreactor is a cartridge that contains [of] a multitude of semi-permeable tube-shaped fibers encased in a hollow shell. The terms hollow fiber reactor and hollow fiber bioreactor are used interchangeably. A preferred device for methods is that described in copending, allowed, U.S. application Serial No. 08/506,173.

Please amend the paragraphs on page 28, lines 6-19, as follows;

While Th2 clones have been used in adoptive transfer studies in animals, regulatory cells, including Th1 and Th2 cells, have not been used in ACT protocols in humans. Such protocols are limited by the inability to differentiate and produce therapeutically effective quantities of such regulatory cells. The methods herein, however, provide a means to produce such clinically relevant quantities of cells, and, thereby provide a means to ameliorate disorders, provide vaccines, and suppress tissue or organ rejection. The methods herein also provide a means to produce clinically relevant quantities of [relulatory] regulatory and effector cells in the absence of IL-2.

Also provided herein, are methods for growing cells that are therapeutically useful for treatment of HIV infection, including treatment of A.I.D.S. by [enchancing] enhancing or restoring the immune system (see, e.g., Examples 3 and 4).

[[3]]

[[4]]

U.S.S.N 09/824,906
GRUENBERG
PRELIMINARY AMENDMENT ATTACHMENT

COPY

Please amend the paragraph on page 37, lines 4-17, as follows;

Artificial kidney cartridges (CD Medical of Hialeah, FL) having a length of 14 inches, an ECS [volume of] volume of 120 ml, and a molecular weight cutoff (MWC) of 6,000 daltons were selected as the hollow fiber bioreactors for use in the hollow fiber processing apparatus. To ensure equal distribution of nutrients across the entire length of these low MWC cartridges, an automatic on/off solenoid valve was placed on the outflow opening of the bioreactor. When the solenoid is in the "off' position, medium is prevented from exiting the bioreactor. Instead, the medium ultrafiltrates to the cells in the ECS equally to all points of the bioreactor. The medium then passes out of the bioreactor through the ports. Ultrafiltration of nutrients is more physiological and therefore more desirable for maintenance of dense cultures of cells (see, e.g., Swaab et al. (1974) Cancer Res. 34:2814; and Davis et al. (1974) Chem. Eng. J. 7:213).

Please amend the paragraph on page 38, lines 10-12, as follows;

In preferred embodiments, mitogenic monoclonal antibodies are coated onto the hollow fiber [surafce] surface in order to deliver the proper signals necessary to cause the immune cells to divide.

Please amend the paragraph on page 41, lines 18-28, as follows;

The compositions of cell can be administered by any suitable means, including, but not limited to, intravenously, parenterally, or locally. The particular mode selected will depend upon the particular treatment and trafficking of the cells. Intravenous administration is presently preferred. Typically, about 10^{10}-10^{11} cells can be administered in a volume of a 50 ml to 1 liter, preferably about 50 ml to 250 ml., more preferably about 50 ml to 150 ml, and most preferably about 100 ml. The volume will depend upon the disorder treated and the route of [adminstration] administration. The cells may be administered in a single dose or in several doses over selected time intervals in order to titrate the dose, particularly when restoration of immune system balance is the goal.

Please amend the paragraph on page 45, lines 18-22, as follows;

Th1-mediated autoimmune diseases, such as, but not limited to, autoimmune thyroid diseases, anti-tubular basement membrane disease (kidney) Sjögren's syndrome, ankylosing [spohdylitis] spondylitis, ureoretinitis and others, can be treated by administration of compositions containing a clinically relevant, typically 10^9-10^{11}, Th2 cells or a Th2-like composition.

Please amend the paragraph on page 47, lines 16-28, as follows;

Other infectious diseases that can be treated with Th1 cell compositions include, but are not limited to: influenza viruses, polio virus, leukemia viruses, hepatitis viruses, respiratory [synctial] syncytial virus, herpes viruses, retroviruses Epstein-Barr virus, [syphillis] syphylis (Treponema pallidum), cutaneous T-cell lymphoma (mycosis fungoides), Rhodococcus equi (intracellular respiratory pathogen), hypersensitivity pneumonitis, onchocercal keratitis (river blindness), burn victims, chlamydia trachomatis, mycobacterium avium, candida albicans, coxackievirus, Leishmania major infection, cryptococcal infection and Bordetella pertussis respiratory infection.

Infectious diseases that can be treated with Th2 cell compositions include, but are not limited to: filarial nematode (parasite), Plasmodium [chaboudi] chaboudi (malaria), and Borrelia burgdofi (spriochete) infections.

Please amend the paragraphs on page 48, lines 6-24, as follows;

Th1 cells can also be used to mediate tumor regression in cancers including melanoma, breast cancer, head and neck cancer, prostate cancer and lung cancer. [These] There is evidence that for certain tumors, a Th2 [rsponse] response may mediate regression.

6. Vaccination

The development of effective vaccine strategies for intracellular pathogens, including, but not limited to, bacteria, viruses and parasites, is one of the major frontiers of medical research. Research centers on antigens from pathogenic organisms and adjuvants that can elicit a Th1-like response in

patients. It is known that a Th1 response is protective for infectious pathogens. Th1 responses are weak or non-existent in some patients with most vaccine protocols. Other research focuses on eliciting an IgA antibody response, which is thought to be protective against organisms that enter the body through [muscous] mucous membranes. An IgA response is mediated by Th2 cells. To better control the type of immune response a patient will elicit to a vaccine, the methods herein provide a means for ex vivo vaccination (i.e., the addition of the vaccine antigen(s) to patient mononuclear cells ex vivo, whereby [thecells] the cells are activated under conditions that promote the desired regulatory cell differentiation.

Please amend the paragraph on page 53, lines 21-28, as follows;

The purified CD4+ cells were divided into [twoeparate] two separate groups of 1 million cells each. The first group was activated with immobilized anti-CD3 mAb in the presence of 400 U/ml of IL-4 and 10 μg/ml of anti-IFN-γ mAb and anti-CD28 mAb. This first group (Th2) was expanded under these conditions for another 10 days. The second group was activated with immobilized anti-CD3 in the presence of 25 U/ml of IL-12 and 150 U/ml of IFN-γ, and anti-CD28 mAb. These cells were harvested and washed after 6 days of culture.

WHAT IS CLAIMED IS:

1. (Amended) A method for selectively stimulating proliferation and differentiation of T lymphoid cells to generate a high density of clinically relevant numbers of T lymphoid cells, comprising:

collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;

treating the cells [are] under conditions whereby *ex vivo* differentiation of the cells into Th2-like or Th2 cells is induced; and

contacting, in the absence of exogenous interleukin-2, the material with two or more activating proteins specific for cell surface proteins present on cells

in the material and in an amount sufficient to induce *ex vivo* cell expansion, whereby the cells expand to at least about 10^{10} cells comprising predominantly Th2 or Th2-like cells.

6. (Amended) The method of claim 1, wherein the immune cells are activated ex vivo in the presence of [interferon-γ] anti-gamma interferon, whereby differentiation of Th2 cells are effected.

79. (Amended) The method of claim 74, wherein the disease is rheumatoid arthritis, wherein the composition is produced by a method comprising:

collecting mononuclear cells from a rheumatoid arthritis patient;

expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to suppress or reduce the chronic inflammatory lesions of the arthritis is generated; and

infusing the resulting composition of cells into the patient.

82. (Amended) The method of claim 74, wherein the disease is multiple sclerosis, and the composition is produced by a method, comprising:

collecting mononuclear cells from a multiple sclerosis patient;

expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to ameliorate the symptoms or retard or stop the progression of multiple sclerosis is generated; and

infusing the resulting composition of cells into the patient.

87. (Amended) The method of claim 74, wherein the disease is an inflammatory bowel disease (IBD), and the composition is produced by a method, comprising:

collecting mononuclear cells from an IBD patient;

expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to ameliorate the symptoms or retard or stop the progression of the IBD; and

infusing the resulting composition of cells into the patient.

U.S.S.N 09/824,906
GRUENBERG
PRELIMINARY AMENDMENT ATTACHMENT

91. (Amended) The method of claim 87, wherein the Th2 cells [are] express integrin, $\alpha 4$, $\beta 7$.

92. (Amended) A method for suppression of transplant rejection, comprising:

collecting mononuclear cells from a patient prior to undergoing organ or tissue transplantation;

expanding the cells under conditions whereby a composition containing an amount of Th2 cells sufficient to prevent rejection of the transplanted organ or tissue is generated; and

infusing the resulting composition of cells into the patient.

* * *

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: _____
Stephanie L. Seidman
Registration No. 33,779

Attorney Docket 24731-500G
Address all correspondence to:
HELLER EHRMAN WHITE & McAULIFFE LLP
4350 La Jolla Village Drive, Suite 600
San Diego, CA 92122-1246
Telephone: (858) 450-8400
Facsimile: (858) 587-5360
EMAIL: sseidman@hewm.com